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EFFECTS OF NITRIC OXIDE PRODUCTION ON GLUTATHIONE LEVELS IN AN ANIMAL MODEL OF DEPRESSION

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Abstract

Stimulation of glutamate receptors induces neuronal nitric oxide (NO) release, which in turn modulates glutamate transmission. The present study evaluated the effects of acute, chronic or combined stress on NO production *via* the accumulation of nitrite, the stable metabolite of NO, in the prefrontal cortex and hippocampus of male Wistar rats. Given that glutathione (GSH) plays a critical role in protecting cells from oxidative stress, as well as maintaining the thiol redox state, GSH levels in cytosolic fractions of both brain structures were examined. A significant increase in nitrite levels was obtained after 3 weeks of chronic isolation stress, followed by combined stresses (chronic + acute stress). Moreover, GSH levels were significantly decreased after chronic and both combined stresses in both brain structures. Our data support the idea that GSH might represent an important buffer of NO toxicity in the brain, indicating that compromised redox buffering controlled by GSH makes neuronal cells susceptible to endogenous physiological flux of NO.

Introduction

Nitric oxide (NO) has been considered as an important neurotransmitter involved in the pathophysiology of depression. A free radical gas molecule, NO is synthesized from L-arginine by the enzyme NO synthase (NOS). In the brain, NO production can occur as a result of activity of either neuronal nNOS, as a dominant constitutive form, and inducible iNOS which has been associated with pathological processes [1]. NO plays a crucial role in synaptic plasticity, neuromodulation and other physiological functions in the brain, while under pathophysiological conditions, it may induce oxidative damage. Overproduction of NO production may lead to alterations of neuronal signaling and to cell damage through the cytotoxicity of NO oxidation derivatives. Chemical inactivation of NO by reaction with oxygen, superoxide, and glutathione (GSH) competes with specific interactions with target proteins. Therefore, our goal was to examine the effects of acute, chronic or combined stresses on NO production in cytosolic fractions of hippocampus and prefrontal cortex of male Wistar rats. Since GSH helps protect brain cells from nitric oxide-peroxynitrite damage, we studied the effects of all stress groups on GSH levels in both brain structures.

Experimental

Adult male Wistar rats, aged three months, were divided at random into four groups: I-controls; II-acute stresses i.e. 2 h of immobilization (IM) or cold (C) stress (4°C); III-3 weeks of chronic social isolation (IS) as an animal model of depression; IV-combined stresses (IS+IM, IS+C) i.e. rats undergo IS stress followed by a single exposure to 2 h of either IM or C stress. Cytosolic NO levels in prefrontal cortex (PFC) and hippocampus (HIPP) were estimated from the amounts of nitrite/nitrate levels (nM/mg of protein) with the colorimetric assay using Griess reagent, whereby nitrates were previously transformed into nitrites by Cd reduction. Briefly, nitrite production was determined by mixing 50 µL of the assay buffer with 50 µL of Griess reagent (1.5 % sulfanilamide in 1 M HCl plus 0.15 % N-(1-naphthyl) ethylenediamine dihydrochloride in distilled water, v:v). Nitrite content was calculated from maximum absorbance (550 nm) using the sodium nitrite (µM) standard curve. GSH levels were quantified from fresh prepared cytosolic fractions of prefrontal cortex and hippocampus and estimated according to the method of Ellman. Data were analyzed by two-way ANOVA followed by the Duncan post-hoc test.

Results and discussion

The effects of acute, chronic or combined stress on NO metabolites (nitrate/nitrite) and GSH levels are presented in Fig.1.

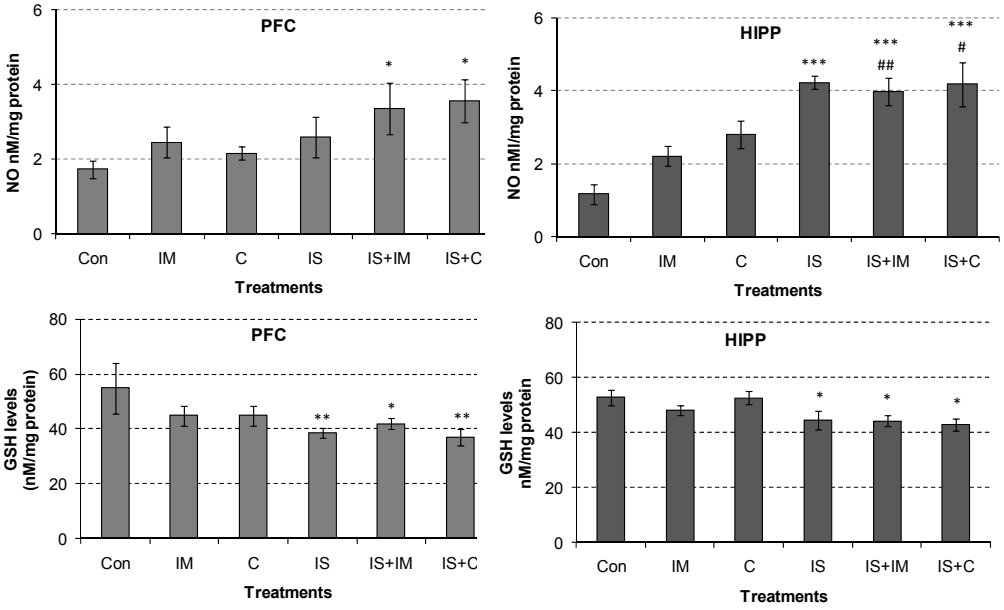

Figure1. Effect of acute (immobilization IM or cold C) stress, chronic isolation (IS) or their combination (IS+IM and IS+C) on nitrite/nitrate levels as well as total GSH in the prefrontal cortex (PFC) and hippocampus (HIPP). Values are mean ± SEM of 6 animals for each group. ***Compared between stressed animals to the control group ($p < 0.001$); combined stress *vs.* acute stress ($^{\#\#\#}p < 0.001$)

Significant increase of nitrite content following acute C and IS stress in HIPP ($p<0.01$; $p<0.001$, respectively) was found, while it remained unchanged in the PFC. We previously reported increased nNOS protein expression in all stress groups in PFC and HIPP, while elevated iNOS expression following chronic IS and combined stress was observed only in PFC [2]. Given that elevation of NO due to increased expression of nNOS after acute stress acts predominantly as a neuromodulator by decreasing glutamate release, the observed increase of hippocampal nitrite level under acute C stress could underlie a normal physiological and protective role [3]. No influence was observed in GSH levels following both acute stresses. Moreover, sustained overproduction of nitrite levels observed following chronic IS and both combined stresses ($p<0.001$ and $p<0.05$) compared to the control or acute stress alone ($^{\#\#}p<0.01$; $^{\#}p<0.05$), indicate prooxidant states in both brain structures. Given that GSH is a major antioxidant capable of scavenging hydrogen peroxide and peroxynitrite, while undergoing oxidation to glutathione disulphide, depletion of GSH, following chronic isolation stress, could be the result of decreased detoxification of peroxynitrite, suggesting that it may be chronically produced by NO overproduction.

Conclusions

Based on the above results, it could be concluded that 3 weeks of chronic social isolation causes NO overproduction in both brain structures causing oxidative/nitrosative stress. Moreover, chronic IS stress may impose a more prominent pro-oxidative condition [4], which may be responsible for GSH consumption, possibly overcoming the capacity of GSH synthesis. Further studies are needed to elucidate the precise mechanisms underlying GSH protection against chronically-induced oxidative stress.

Acknowledgement

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